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(71) Applicant *(for all designated States except US)*: MEDAREX, INC. [US/US]; 22 Chambers Street, Princeton, NJ 08542 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : TEMPEST, Philip, R. [GB/GB]; 63 Brighton Place, Aberdeen AB1 6RT (GB). HARRIS, William, J. [GB/GB]; 3 Caesar Avenue, Carnoustie DD7 6DR (GB). CARR, Frank, J. [GB/GB]; Birchlea, The Holdings, Balmedie, Aberdeenshire AB23 8XU (GB).

(74) Agents: MANDRAGOURAS, Amy, E. et al.; Lahive & Cockfield, 60 State Street, Boston, MA 02109 (US).

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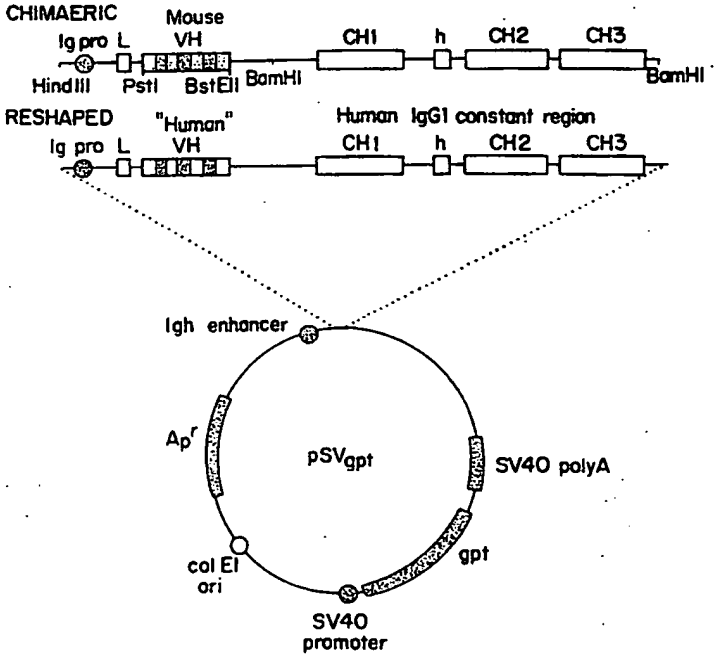

(57) Abstract

Humanized antibodies are described which are specific to an Fc receptor (FcR). The humanized antibodies have at least a portion of a complementarity determining region (CDR) derived from a non-human antibody, e.g. murine, with the remaining portions being human in origin. The use of humanized antibodies rather than murine antibodies in human therapy should alleviate some of the problems associated with the use of some murine monoclonal antibodies because only the substituted CDRs will be foreign to a human's immune system. The humanized antibodies can be used in the same manner as their murine counterparts.

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HUMANIZED ANTIBODIES TO Fc RECEPTORS FOR IMMUNOGLOBULIN G ON HUMAN MONONUCLEAR PHAGOCYTES

Background

Human Fcγ receptors (FcγR) (reviewed in Fanger, M.W., et al. (1989) Immunology Today 10:92-99), of which there are three structurally and functionally distinct types (i.e., FcγRI, FcγRII and FcγRIII), are well-characterized cell surface glycoproteins that mediate phagocytosis or antibody-dependent cell cytotoxicity (ADCC) of immunoglobulin G (IgG) opsonized targets. Antibodies have been made which are directed towards FcγR for various purposes, e.g., targeting of immunotoxins to a particular target cell type, or radioimaging a particular target cell type. The antibodies typically have been murine antibodies.

Murine monoclonal antibodies are sometimes desirable for human therapeutic applications because the antibodies can be purified in large quantities and are free of contamination by human pathogens such as the hepatitis or human immunodeficiency virus. Murine monoclonal antibodies have been used in some human therapies, however, results have not always been desirable due to the development of an immune response to the "foreign" murine proteins. The immune response has been termed a human anti-mouse antibody or HAMA response (Schroff, R., et al. (1985), Cancer Res., 45, 879-885) and is a condition which causes serum sickness in humans and results in rapid clearance of the murine antibodies from an individual's circulation. The immune response in humans has been shown to be against both the variable and the constant regions of the murine immunoglobulin.

Recombinant DNA technology has provided the ability to alter antibodies by substituting specific immunoglobulin regions from one species with immunoglobulin regions from another species. Neuberger et al. (Patent Cooperation Treaty Patent Application No. PCT/GB85/00392) describes a process whereby the complementary heavy and light chain variable domains of an Ig molecule from one species may be combined with the complementary heavy and light chain Ig constant domains from another species. This process may be used to substitute the murine constant region domains to create a "chimeric" antibody which may be used for human therapy. A chimeric antibody produced as described by Neuberger et al. would have the advantage of having the human Fc region for efficient stimulation of antibody mediated effector functions, such as complement fixation, but would still have the potential to elicit an immune response in humans against the murine ("foreign") variable regions.

Winter (British Patent Application Number GB2188538A) describes a process for altering antibodies by substituting the complementarity determining regions (CDRs) with those from another species. This process may be used to substitute the CDRs from the murine variable region domains of a monoclonal antibody with desirable binding properties

(for instance to a human pathogen) into human heavy and light chain Ig variable region domains. These altered Ig variable regions may then be combined with human Ig constant regions to create antibodies which are totally human in composition except for the substituted murine CDRs. The "reshaped" or "humanized" antibodies described by Winter elicit a considerably reduced immune response in humans compared to chimeric antibodies because of the considerably less murine components. Further, the half life of the altered antibodies in circulation should approach that of natural human antibodies. However, as stated by Winter, merely replacing the CDRs with complementary CDRs from another antibody which is specific for an antigen such as a viral or bacterial protein, does not always result in an altered antibody which retains the desired binding capacity. In practice, some amino acids in the framework of the antibody variable region interact with the amino acid residues that make up the CDRs so that amino acid substitutions into the human Ig variable regions are likely to be required to restore antigen binding.

Summary of the Invention

The present invention pertains to humanized antibodies specific to an Fc receptor (FcR). The humanized antibodies have at least a portion of a complementarity determining region (CDR) derived from a non-human antibody, e.g., murine, with the remaining portions being human in origin. The use of humanized antibodies rather than murine antibodies in human therapy should alleviate some of the problems associated with the use of some murine monoclonal antibodies because only the substituted CDRs will be foreign to a human host's immune system.

The present invention further pertains to the use of humanized antibodies specific to an FcR as components in heteroantibodies, bifunctional antibodies, or immunotoxins. The humanized antibody specific to an FcR may be used in the same manner and for the same purpose as its corresponding murine counterpart. For example, the humanized anti-Fc receptor antibody of this invention can be used to treat cancer, allergies, and infectious and autoimmune diseases. Diagnostic applications of the antibodies include their use in assays for FcRI levels and assays for substances that influence FcR levels.

Brief Description of the Drawings

Figure 1 compares the amino acid sequences of murine 022 VH (SEQ ID NO:3) with the amino acid sequences of humanized NEWM-based VH (022 NMVH) (SEQ ID NO:1) and humanized KOL-based VH (022 KLVH) (SEQ ID NO:2). The CDRs are boxed. Murine residues retained in the human portion are indicated by the inverted black triangles.

Figure 2 compares the amino acid sequences of murine 022 VK (SEQ ID NO:28) with humanized REI-based VK (022 HuVK) (SEQ ID NO:4). The CDRs are boxed. Murine residues retained in the human portion are indicated by the inverted black triangles.

Figure 3 depicts the vector used for expression of the humanized or chimeric 022 heavy chain gene.

Figure 4 depicts the vector used for expression of the humanized or chimeric 022 kappa chain gene.

Figure 5 depicts the binding of the test antibodies in the enzyme liked immunoassay described in the example.

Detailed Description

The present invention pertains to a humanized antibody specific for an Fc receptor. The humanized antibody is made up of a human antibody having at least a portion of a complementarity determining region (CDR) derived from a non-human antibody. The portion is selected to provide specificity of the humanized antibody for a human Fc receptor. The humanized antibody has CDR's derived from a non-human antibody and the remaining portions of the antibody molecule are human.

The antibody may be a complete antibody molecule having full length heavy and light chains or any fragment thereof, e.g., Fab or $(Fab')_2$ fragment. The antibody further may be a light chain or heavy chain dimer, or any minimal fragment thereof such as a Fv or a single chain construct as described in Ladner et al. (U.S. Patent No. 4,946,778, issued August 7, 1990), the contents of which is expressly incorporated by reference.

The human antibody of the present invention may be any human antibody capable of retaining non-human CDRs. The preferred human antibody is derived from known proteins NEWM and KOL for heavy chain variable regions (VHs) and REI for Ig kappa chain, variable regions (VKs). These proteins are described in detail in the examples below.

"Complementarity determining region" (CDR) is an art recognized terminology and the technique used for locating the CDRs within the described sequences also is conventional.

The portion of the non-human CDR inserted into the human antibody is selected to be sufficient for allowing binding of the humanized antibody to the Fc receptor. A sufficient portion may be selected by inserting a portion of the CDR into the human antibody and testing the binding capacity of the created humanized antibody using the enzyme linked immunosorbent assay (ELISA) described in the examples below.

All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the

humanized antibody to the Fc receptor. The exemplified non-human CDR is derived from a murine antibody, particularly the CDR is derived from a monoclonal antibody (mab), mab 22. The mab 22 antibody is specific to the Fc receptor and further is described in U.S patent application Serial No. 07/151,450, filed on February 2, 1988, and in Fanger et al. (U.S. Patent No. 4,954,617, issued September 4, 1988). The entire contents of the aforementioned pending application and issued patent are expressly incorporated by reference.

The CDRs are derived from a non-human antibody specific for a human Fc receptor. The CDRs can be derived from known Fc receptor antibodies such as those discussed in the Fanger et al. patent application and issued patent cited above (hereinafter Fanger et al.). The CDR may be derived from an antibody which binds to the Fc receptor at a site which is not blocked by human immunoglobulin G. The antibody also may be specific for the high affinity Fc receptor for human immunoglobulin G. Examples of antibodies from which the non-human CDRs may be derived are mab 32, mab 22, mab 44, mab 62, mab 197 and anti-FcRI antibody 62. The humanized mab 22 antibody producing cell line has been deposited at the American Type Culture Collection on November 4, 1992 under the designation HA022CL1 and has the accession no. CRL 11177.

The present invention also pertains to bifunctional antibodies or heteroantibodies having at least one humanized antigen binding region derived from a humanized anti-Fc receptor antibody and at least one antigen binding region specific for a target epitope. The humanized antigen binding region may be derived from a humanized anti-Fc receptor antibody as described above. Bifunctional and heteroantibodies having an antibody portion specific for an Fc receptor are described in detail by Fanger et al.

It should be understood that the humanized antibodies of the present invention may be used in the same manner, e.g., as components of immunotoxins or heteroantibodies, as their corresponding non-humanized counterparts described by Fanger et al. The humanized antibodies further share the same utilities as their non-humanized counterparts. All aspects of the teachings of the Fanger et al. application and patent are incorporated by reference.

The humanized antibody of the present invention may be made by any method capable of replacing at least a portion of a CDR of a human antibody with a CDR derived from a non-human antibody. Winter describes a method which may be used to prepare the humanized antibodies of the present invention (UK Patent Application GB 2188638A, filed on March 26, 1987), the contents of which is expressly incorporated by reference. The human CDRs may be replaced with non-human CDRs using oligonucleotide site-directed mutagenesis as described in the examples below.

The humanized antibody of the present invention may be made as described in the brief explanation below. A detailed method for production is set forth in the examples. It should be understood that one of ordinary skill in the art may be able to substitute known

conventional techniques for those described below for the purpose of achieving the same result. The humanized antibodies of the present invention may be produced by the following process:

(a) constructing, by conventional techniques, an expression vector containing an operon with a DNA sequence encoding an antibody heavy chain in which the CDRs and such minimal portions of the variable domain framework region that are required to retain antibody binding specificity are derived from a non-human immunoglobulin, and the remaining parts of the antibody chain are derived from a human immunoglobulin, thereby producing the vector of the invention;

(b) constructing, by conventional techniques, an expression vector containing an operon with a DNA sequence encoding a complementary antibody light chain in which the CDRs and such minimal portions of the variable domain framework region that are required to retain donor antibody binding specificity are derived from a non-human immunoglobulin, and the remaining parts of the antibody chain are derived from a human immunoglobulin, thereby producing the vector of the invention;

(c) transfecting the expression vectors into a host cell by conventional techniques to produce the transfected host cell of the invention; and

(d) culturing the transfected cell by conventional techniques to produce the altered antibody of the invention.

The host cell may be cotransfected with the two vectors of the invention, the first vector containing an operon encoding a light chain derived polypeptide and the second vector containing an operon encoding a heavy chain derived polypeptide. The two vectors contain different selectable markers, but otherwise, apart from the antibody heavy and light chain coding sequences, are preferably identical, to ensure, as far as possible, equal expression of the heavy and light chain polypeptides. Alternatively, a single vector may be used, the vector including the sequences encoding both the light and the heavy chain polypeptides. The coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both.

The host cell used to express the altered antibody of the invention may be either a bacterial cell such as Escherichia coli, or a eukaryotic cell. In particular a mammalian cell of a well defined type for this purpose, such as a myeloma cell or a Chinese hamster ovary cell may be used.

The general methods by which the vectors of the invention may be constructed, transfection methods required to produce the host cell of the invention and culture methods required to produce the antibody of the invention from such host cells are all conventional techniques. Likewise, once produced, the humanized antibodies of the invention may be purified according to standard procedures of the art, including cross-flow

filtration, ammonium sulphate precipitation, affinity column chromatography, gel electrophoresis and the like.

It should be understood that the humanized antibodies of this invention perform in a manner which is the same or similar to that of the non-humanized versions of the same antibodies. It also is noted that the humanized antibodies of this invention may be used for the design and synthesis of either peptide or non-peptide compounds (mimetics) which would be useful for the same therapy as the antibody (Saragobi et al., Science 253:792-795 (1991)), the contents of which is expressly incorporated by reference.

The following examples are provided as a further illustration of the present invention and should in no way be construed as being limiting.

EXAMPLES

In the following examples all necessary restriction and modification enzymes, plasmids and other reagents and materials were obtained from commercial sources unless otherwise indicated.

In the following examples, unless otherwise indicated, all general recombinant DNA methodology was performed as described in "Molecular Cloning, A Laboratory Manual" (1982) Eds T. Maniatis et al., published by Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, the contents of which is expressly incorporated by reference.

In the following examples the following abbreviations were employed:

dCTP	deoxycytidine triphosphate
dATP	deoxyadenosine triphosphate
dGTP	deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
DTT	dithiothreitol
C	cytosine
A	adenine
G	guanine
T	thymine
PBS	phosphate buffered saline
PBST	phosphate buffered saline containing 0.05% Tween 20 (pH 7.5)

Example 1 - Production of Humanized Antibodies Specific for an Fc Receptor

The source of the donor CDRs used to prepare the humanized antibody was a murine monoclonal antibody, mab 22, which is specific for the Fc receptor. A mab 22

hybridoma cell line (022WCL-1) was established. Cytoplasmic RNA was prepared from the mab 22 cell line using the method described by Favoloro et al. (Methods in Enzymology 65, 718-749 (1980)), the contents of which is expressly incorporated by reference. The cDNA was synthesized using IgGI and kappa constant region primers. The primer CG1FOR was used for the heavy chain variable (VH) region and the primer CK2FOR was used for the Ig kappa chain variable region (VK). The cDNA synthesis reactions mixtures consisted of 1 μg RNA, 0.5μM CG1FOR or CK2FOR, 250 μM each of dATP, dCTP, dGTP, and dTTP, 50 mM Tris HCl (pH 7.5), 75 mM KC1, 10 mM dithiothreitol, 3 mM $MgC1_2$ and 20μ RNAguard (sold by Pharmacia, Milton Keynes, U.K.) in a total volume of 50 μl. The samples were heated at 72°C for two minutes and slowly cooled to 37°C. Murine moloney leukemia virus reverse transcriptase (100 μl - sold by Life Technologies, Paisley, U.K.) was added to the samples and the transcriptase containing samples were incubated at 42°C for sixty minutes.

VH and VK cDNAs were then amplified using the polymerase chain reaction (PCR) as described by Saiki et al. (Science 239, 487-491 (1988)), the contents of which is expressly incorporated by reference. The primers used in the above steps were as follows:

CG1FOR (SEQ ID NO:5) 5' GGAAGCTTAGACAGATGGGGGTGTCGTTTTG 3'
VH1FOR (SEQ ID NO:6) 5' TGAGGAGACGGTGACCGTGGTCCCTTGGCCCCAG 3'
VH1BACK (SEQ ID NO: 7) 5' AGGTSMARCTGCAGSAGTCWGG 3'
SH1BACK (SEQ ID NO:8) 5' TGGAATTCATGGRATGGAGCTGGRTCWTBHTCTT 3'
SH2BACK (SEQ ID NO:9) 5' TGGAATTCATGRACTTCDGGYTCAACTKRRTTT 3'

CK2FOR (SEQ ID NO:10) 5' GGAAGCTTGAAGATGGATACAGTTGGTGCAGC 3'
VK1BACK (SEQ ID NO:11) 5' GACATTCAGCTGACCCAGTCTCCA 3'
VK5BACK (SEQ ID NO:12) 5' TTGAATTCGGTGCCAGAKCWSAHATYGTKATG 3'
VK6BACK (SEQ ID NO:13) 5' TTGAATTCGGTGGCAGAKCWSAHATYGTKCTC 3'
VK7BACK (SEQ ID NO:14) 5' TTGAATTCGGAGCTGATGGGAACATTGTAATG 3'

Restriction sites incorporated in primers to facilitate cloning are underlined.

The PCR amplification of murine Ig DNA was conducted using the methodology described by Orlandi et al. (Proc. Natl. Acad. Sci USA 86, 3833-3838 (1989), the contents of which is expressly incorporated by reference. The DNA/primer mixtures consisted of RNA/cDNA hybrid (10 μl) and 25pmol each of CG1FOR and SH1BACK or SH2BACK for PCR amplification of VH. The DNA/primer mixtures consisted of RNA/cDNA hybrid (10 μl) and 25pmol each of CK2FOR and VK1BACK, VK5BACK, VK6BACK, VK7BACK for PCR amplification of VK. dATP, dCTP, dGTP and dTTP (250

μM each), 10mM Tris HCl (pH 8.3), 60mM KCl, 1.5mM $MgCl_2$, 0.01% (w/v) gelatin, 0.01% (v/v) Tween 20, 0.01% (v/v) NP40 and 2.5μ Amplitaq (sold by Cetus, Beaconsfield, U.K.) were added to the samples in a final volume of 50 μl. The samples were subjected to 25-30 thermal cycles of PCR at 94°C for thirty seconds, 55°C for thirty seconds, 72°C for one minute and a final cycle at 72°C for five minutes.

The amplified VH and VK DNAs were run on a low melting point agarose gel and purified by Elutip-d column chromatography (sold by Schleicher and Schueell, Anderman, Walton, U.K.) for cloning and sequencing. The purified VH DNAs were cut with Eco I or Pst I and Hind III and cloned into M13mp18 and mp19 (sold by Pharmacia, Milton Keynes, U.K.). The purified VK DNAs were cut with Pvu II or Eco I and Hind III and cloned into M13mp18 and mp19. For general cloning methodologies see Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)), the contents of which are expressly incorporated by reference. The resulting collection of clones were sequenced by the dideoxy method using T7 DNA polymerase (sold by Pharmacia, Milton Keynes, U.K.) as described by Sanger et al. (Proc. Natl. Acad. Sci. USA 74, 5463-5467, (1979)), the contents of which are expressly incorporated by reference.

From the sequences of the O22 VH and VR domains the CDR sequences were determined with reference to the database of Kabat et al. ("Sequences of Proteins of Immunological Interest" US Department of Health and Human Services, US Government Printing Office), the contents of which is expressly incorporated by reference, and utilizing computer assisted alignment with other VH and VK sequences.

Transfer of the murine O22 CDRs to human frameworks was achieved by oligonucleotide site-directed mutagenesis as described by Nakamye et al. (Nucleic Acids Res 14, 9679-9687 (1986)), the contents of which is expressly incorporated by reference. The primers used were as follows:

KLVHCDR1 (SEQ ID NO:15): 5' TGCCTGTCTCACCCAATACATGTAA
TTGTCACTGAAATGAAGCCAGACGMGGAGCGGACAG

KLVHCDR2 (SEQ ID NO:16): 5' TGTAAATCTTCCCTTCACACTGTCTGGATAGTA
GGTGTAACTACCACCATCACTAATGGTTGCAACCCACTCAGG

KLVHCDR3 (SEQ ID NO:17): 5' GGGGTCCCTTGGCCCCAGTAGTCCATAGC
CCCCTCGTACCTATAGTAGCCTCTTGCACAAAAATAGA

NMVHCDR1 (SEQ ID NO:18): 5' TGGCTGTCTCACCCAATACATGTAATTGT
CGCTGAAAATGAAGCCAGACACGGTGCAGGTCAGGCTCA

NMVHCDR2 (SEQ ID NO:19): 5' TTGCTGGTGTCTCTCAGCATTGTCACTCTC
CCCTTCACACTGTCTGGATAGTAGGTGTAACTACCACCA
TCACTAATGGTTCCAATCCACTCAA

NMVHCDR3 (SEQ ID NO:20): 5' AGACGGTGACCAAGGACCCTTGGCCCCAG
TAGTCCATAGCCCCCTCGTACCTATAGTAGCCTCTTGCACAATAATAG

HuVKCDR1 (SEQ ID NO:21): 5' CTTCTGCTGGTACCAGGCCAAGTAGTTCTTC
TGATTTGAACTGTATAAAACACTTTGACTGGACTTACAGGTGATGGTCAC

HuVKCDR2 (SEQ ID NO:22): 5' GCTTGGCACACCAGATTCCCTAGTGGATG
CCCAGTAGATCAGCAG

HuVKCDR3 (SEQ ID NO:23): 5' CCTTGGCCGAACGTCCACGAGGAGAGGTAT
TGATGGCAGTAGTAGGTGG

The primer for NMVHCDR1 was extended to include a change of NEWM residues Ser 27 Thr 28 to Phe 27 Ile 28. The primer for NMVHCDR2 was extended to include a change of NEWM residue Val 71 to Arg 71.

The DNA templates used for mutagenesis of VHs comprised human framework regions from the crystallographically solved protein NEW described by Saul et al. (J. Biol. Chem. 53, 585-597 (1978)) or KOL described by Schmidt et al. (Z. Physical Chem. 364, 713-747 (1983)). The DNA templates used for mutagenesis of VKs comprised human framework regions from the crystallographically solved protein REI described by Epp et al. (Eur. J. Biochem. 45, 513-524 (1974)). The contents of each of the forementioned references are expressly incorporated by reference.

M13 based templated M13VHPCR1 (for NEWMVH), M13VHPCR2 (for KOLVH) and M13VKPCR2 (for REIVK) comprising human frameworks with irrelevant CDRs were prepared as described by Riechmann et al. (Nature 332, 323-327 (1988)), the contents of which are expressly incorporated by reference. Oligonucleotide site-directed mutagenesis was carried out using the following protocol. A 5-fold molar excess of each phosphorylated mutagenic oligonucleotide was added along with the universal M13 sequencing primer (5'- GTAAAACGACGGCCAGT) (SEQ ID NO:24). All of the primers were annealed in 20ul 0.1M TrisHCl (pH8.0) and 10mM $MgCl_2$ by heating to 70-85°C for two minutes and slowly cooling to room temperature. 10 mM DTT, 1 mM ATP, 40 μM each of dATP, dCTP, dGTP and dTTP, 2.5μ T7 DNA polymerase (sold by United States

Biochemicals) and 0.5μ T4DNA ligase (sold by Life Technologies, Paisley, U.K.) was added to the annealed DNA in a reaction volume of 30μl and incubated at 22° - 37°C for one to two hours. The newly extended and ligated strand was preferentially amplified over the parental strand in a thermostable DNA polymerase directed reaction using the M13 reverse sequencing primer (5' AACAGCTATGACCATG) (SEQ ID NO:25). The reverse sequencing primer is not complementary to the parental strand. The reaction mixture of 50μl contained 1 μl extension/ligation product, 25 pmol M13 reverse sequencing primer, 250μM each of dATP, dCTP, dGTP and dTTP, 1μ Vent DNA polymerase (sold by New England Biolabs, Bishop's Stortford, U.K.) or 2.5μ Amplitaq (sold by Cetus, Beaconsfield, U.K.) in the appropriate buffer supplied by the enzyme manufacturer and was subjected to thirty thermal cycles of 94°C, 30s, 55°C, 30s, 75° or 72°C, 90s; ending with 5 min at 72°C. A 4μl aliquot of this sample was then amplified by PCR using both M13 universal and reverse sequencing primers in a reaction mixture of 50μl containing 25 pmol of each primer, 250μM each of dATP, dCTP, dGTP and dTTP, 2.5μ Amplitaq (Cetus) in the buffer supplied by the enzyme manufacturer. Amplified DNAs were digested with HindIII and BamHI and cloned into M13mp19 and sequenced.

Mutagenesis of M13VHPCR2 KOL VH residue Leu71 to Arg71 was by the overlap/extension PCR method of Ho et al. (Gene, 77, 51-55 (1989)), the contents of which is expressly incorporated by reference. The overlapping oligonucleotides used were 5' - TTTACAATATCGAGACAACAGCAA (SEQ ID NO:26) and 5' - TTGCTGTTGTCTCTCGATTGTAAA (SEQ ID NO:27).

The amino acid sequences of the humanized antibodies were compared to the known murine antibodies as shown in Figures 1 and 2. The CDR replaced VH and VK genes were cloned into expression vectors pSVgpt and pSVhyg as shown in Figures 3 and 4 as described by Orlandi et al. (cited supra). The CDR replaced NEWMVH and KOLVH genes together with the Ig heavy chain promoter, appropriate splice sites and signal peptide sequences were excised from M13 by digestion with HindIII and BamHI and cloned into the pSVgpt expression vector containing the murine Ig heavy chain enchancer, the gpt gene for selection in mammalian cells and genes for replication and selection in E. coli. The plasmid also contains a human IgGI constant region as described by Takahashi et al. (Cell 29, 671-675 (1982)). The construction of the kappa chain expression vector was essentially the same except that the gpt gene was replaced by the hygromycin resistance gene and contains a human kappa constant region (Hieter et al., Cell 22, 197-207 (1980)). The contents of each of the forementioned references are expressly incorporated by reference.

Approximately 5μg of each heavy chain expression vector and 10μg of the kappa chain expression vector were digested with PvuI. The DNAs were mixed together, ethanol precipitated and dissolved in 25μl water. Approximately 5-10 x 10^6 NSO cells (from European Collection of Animal Cell Cultures, Porton Down, U.K.) were grown to semi-

confluency in Dulbecco's modified Eagle's medium (DMEM) plus 10% fetal calf serum (Myoclone plus, Gibco, Paisley, Scotland), harvested by centrifugation and resuspended in 0.5ml DMEM together with the digested DNA in a cuvette. After five minutes in ice, the cells were given a single pulse of 170V at 960μF (Gene-Pulser, Bio-Rad, Richmond, CA) and left in ice for a further twenty minutes. The cells were then put into 20ml DMEM + supplemented with 10% FCS and allowed to recover for twenty-four to forty-eight hours. After this time, the cells were distributed into a 24-well plate and selective medium was applied (DMEM, 10% FCS, 0.8μg/ml mycophenolic acid and 250μg/ml xanthine). After three to four days, the medium and dead cells were removed and replaced with fresh selective medium. Transfected clones were visible with the naked eye ten days later.

The presence of human antibody in the medium of wells containing gpt+ transfectants was measured using conventional enzyme linked immunosorbent assay (ELISA) techniques. Wells of a microtitre plate (Immolon, Dynatech, Chantilly, VA) were coated with 100ng goat anti-human IgG antibodies (SeraLab, Crawley Down, U.K.) in 100μl 50mM carbonate buffer pH9.6. After washing with PBST (Phosphate buffered saline pH 7.2 containing 0.05% Tween 20) culture medium in 100μl PBST (5-50ul) was added to each well for one hour at 37°C. The wells were then emptied, washed with PBST and 100μl of 1:1000 dilution peroxidase conjugated goat anti-human kappa constant region antibodies (SeraLab, Crawley Down, U.K.) were added for one hour at 37°C. The wells were emptied, washed with PBST and 100μl OPD substrate buffer (400μg/ml Q-phenylenediamine in 24mM citrate/42mM sodium phosphate pH 5. and 0.0003% (v/v) H_2O_2) was added. The reaction was stopped after a few minutes by the addition of 12.5% H_2SO_4 (25μl) and the absorbance at 492nm was measured.

The antibody secreting cells were expanded and antibody was purified from the culture medium by protein A affinity chromatography as described by Harlow and Lane (Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), the contents of which is expressly incorporated by reference.

The binding of the antibodies to antigen was measured by ELISA. Wells of a microtitre plate (Immunlon 1, Dynatech, Chantilly, VA) were coated with 200ng goat anti-human IgM antibodies (Sera-lab, Crawley Down, U.K.) in 100μl 50mM carbonate buffer pH 9.6 at 37°C for at least one hour. Wells were emptied and washed once with PBST and blocked with 1% BSA in PBS at room temperature for thirty minutes. The wells were emptied and washed with PBST and Cos supernatant containing FcRI/IgM fusion protein was added and incubated for one hour at room temperature. Wells were then emptied and washed three times with PBST and test antibodies diluted in 1% BSA/PBS were added and incubated for one hour at room temperature. In addition, each well contained 2μg human IgG1, lambda antibody (Sigma, Poole, U.K.) The wells were then emptied, washed three times with PBST and 40ng peroxidase goat anti-human kappa constant region antibodies (Sera-Lab, Crawley

Down, U.K.) in 100µl 1% BSA/PBS added to each well. After incubation for one hour at room temperature, the wells were emptied, washed three time with PBST and 10µl HPD substrate buffer was added. The reaction was stopped by the addition of 25µl of 12.5% H_2SO_4 to each well. The absorbance at 492nm was measured and is depicted in Figure 5. The test antibodies were the antibody containing irrelevant CDRs (AA), the fully humanized KOL/REI based antibody (KLVHR/HuVK), the mix and match derivatives of the humanized antibody (KLVHR/MuVK and MuVH/HuVK), the humanized NEWM/REI based antibody (NMVK/HuVK) and the chimeric antibody (MuVH/MuVK).

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: MEDAREX, INC.
(B) STREET: 22 Chambers Street
(C) CITY: Princeton
(D) STATE: New Jersey
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 08542
(G) TELEPHONE: (609)921-7121
(H) TELEFAX: (609)921-7450

(ii) TITLE OF INVENTION: HUMANIZED ANTIBODIES TO Fc RECEPTORS FOR IMMUNOBLOBULIN G ON HUMAN MONONUCLEAR PHAGOCYTES

(iii) NUMBER OF SEQUENCES: 28

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: ASCII text

(v) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB92 23377.4
(B) FILING DATE: 04-NOV-1992
(C) CLASSIFICATION:

(vii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Mandragouras, Amy E.
(B) REGISTRATION NUMBER: 36.207
(C) REFERENCE/DOCKET NUMBER: MXI-013PC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 227-7400
(B) TELEFAX: (617) 227-5941

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1               5                  10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ile Phe Ser Asp Asn
            20                  25                  30

Tyr Met Tyr Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
        35                  40                  45

Gly Thr Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val
    50                  55                  60

Lys Gly Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65                  70                  75                  80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Tyr Tyr Arg Tyr Glu Gly Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Ser Leu Val Thr Val Ser Ser
        115                 120
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Phe Ile Phe Ser Asp Asn
            20                  25                  30

Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Thr Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95
```

Ala Arg Gly Tyr Tyr Arg Tyr Glu Gly Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Pro Val Thr Val Ser Ser
115 120

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Phe Ile Phe Ser Asp Asn
20 25 30

Tyr Met Tyr Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Thr Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Asn Leu Tyr
65 70 75 80

Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Arg Gly Tyr Tyr Arg Tyr Glu Gly Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Ser Val Thr Val Ser Ser
115 120

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 112 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
35 40 45

Ala Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr
65 70 75 80

Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys His Gln
85 90 95

Tyr Leu Ser Ser Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GGAAGCTTAG ACAGATGGGG GTGTCGTTTT G 31

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Thr Gly Ala Gly Gly Ala Gly Ala Cys Gly Gly Thr Gly Ala Cys Cys
1 5 10 15

Gly Thr Gly Gly Thr Cys Cys Cys Thr Thr Gly Gly Cys Cys Cys Cys
20 25 30

Ala Gly

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

AGGTSMARCT GCAGSAGTCW GG 22

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TGGAATTCAT GGRATGGAGC TGGRTCWTBH TCTT 34

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

TGGAATTCAT GRACTTCDGG YTCAACTKRR TTT 33

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGAAGCTTGA AGATGGATAC AGTTGGTGCA GC 32

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GACATTCAGC TGACCCAGTC TCCA 24

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TTGAATTCGG TGCCAGAKCW SAHATYGTKA TG 32

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TTGAATTCGG TGGCAGAKCW SAHATYGTKC TC 32

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

TTGAATTCGG AGCTGATGGG AACATTGTAA TG 32

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 61 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TGCCTGTCTC ACCCAATACA TGTAATTGTC ACTGAAATGA AGCCAGACGM GGAGCGGACA 60

G 61

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TGTAAATCTT CCCTTCACAC TGTCTGGATA GTAGGTGTAA CTACCACCAT CACTAATGGT 60

TGCAACCCAC TCAGG 75

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GGGGTCCCTT GGCCCCAGTA GTCCATAGCC CCCTCGTACC TATAGTAGCC TCTTGCACAA 60

AAATAGA 67

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TGGCTGTCTC ACCCAATACA TGTAATTGTC GCTGAAAATG AAGCCAGACA CGGTGCAGGT 60

CAGGCTCA 68

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TTGCTGGTGT CTCTCAGCAT TGTCACTCTC CCCTTCACAC TGTCTGGATA GTAGGTGTAA 60

CTACCACCAT CACTAATGGT TCCAATCCAC TCAA 94

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

AGACGGTGAC CAAGGACCCT TGGCCCCAGT AGTCCATAGC CCCCTCGTAC CTATAGTAGC 60

CTCTTGCACA ATAATAG 77

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CTTCTGCTGG TACCAGGCCA AGTAGTTCTT CTGATTTGAA CTGTATAAAA CACTTTGACT 60

GGACTTACAG GTGATGGTCA C 81

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GCTTGGCACA CCAGATTCCC TAGTGGATGC CCAGTAGATC AGCAG 45

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

CCTTGGCCGA ACGTCCACGA GGAGAGGTAT TGATGGCAGT AGTAGGTGG 49

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GTAAAACGAC GGCCAGT 17

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

AACAGCTATG ACCATG 16

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

TTTACAATAT CGAGACAACA GCAA 24

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

TTGCTGTTGT CTCTCGATTG TAAA 24

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 112 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Asn Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys His Gln
85 90 95

Tyr Leu Ser Ser Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

CLAIMS

1. A humanized antibody specific to an Fc receptor comprising:
a human antibody having at least a portion of a complementarity determining region derived from a non-human antibody, the portion being selected to provide specificity of the humanized antibody for a human Fc receptor.

2. The antibody of claim 1 wherein the portion is selected to provide specificity to a human Fc receptor such that the humanized antibody formed binds to the Fc receptor at a site which is not blocked by human immunoglobulin G.

3. The antibody of claim 1 wherein the portion is selected to provide specificity of the humanized antibody for the high affinity Fc receptor for human immunoglobulin G.

4. The antibody of claim 1 wherein the portion of a complementarity determining region is derived from a murine antibody.

5. The antibody of claim 1 wherein the portion of a complementarity determining region is derived from a monoclonal antibody selected from the group consisting of mab 32, mab 22, mab 44, mab 62, mab 197, and anti-FcRI antibody 62.

6. The antibody of claim 5 wherein the monclonal antibody is mab 22.

7. The antibody of claim 1 wherein at least a portion of all of the complementarity determining regions of the human antibody are derived from a non-human antibody.

8. The antibody of claim 7 wherein the entire portion of all of the complementarity determining regions of the human antibody are derived from a non-human antibody.

9. The antibody of claim 7 wherein the non-human antibody is mab 22.

10. The antibody of claim 8 wherein the non-human antibody is mab 22.

11. The antibody of claim 1 wherein the human antibody is derived from proteins selected from the group consisting of NEW, KOL, REI, and combinations thereof.

12. A bifunctional antibody or heteroantibody, comprising:

at least one humanized antigen binding region derived from a humanized anti-Fc receptor antibody, and

at least one antigen binding region specific for a target epitope.

13. The antibody of claim 12 wherein the humanized antigen binding region is derived from a humanized anti-Fc receptor antibody selected such that the binding of the humanized antibody to the human Fc receptor is not blocked by human immunoglobulin G.

14. The antibody of claim 13 wherein the humanized anti-Fc receptor antibody is specific for the high affinity Fc receptor for human immunoglobulin.

15. The antibody of claim 12 wherein the humanized antigen binding region has at least a portion of a complementarity determining region derived from a non-human antibody.

16. The antibody of claim 15 wherein the portion of a complementarity determining region is derived from a murine antibody.

17. The antibody of claim 15 wherein the portion of a complementarity determining region is derived from a monoclonal antibody selected from the group consisting of mab 32, mab 22, mab 44, mab 62, mab 197, and anti-FcRI antibody 62.

18. The antibody of claim 17 wherein the monclonal antibody is mab 22.

19. The antibody of claim 15 wherein at least a portion of the all the complementarity determining regions of the human antibody are derived from a non-human antibody.

20. The antibody of claim 19 wherein the entire portion of all of the complementarity determining regions of the human antibody are derived from a non-human antibody.

21. The antibody of claim 19 wherein the non-human antibody is mab 22.

22. The antibody of claim 20 wherein the non-human antibody is mab 22.

23. The antibody of claim 12 wherein the target epitope is that of a cancer cell.

24. The antibody of claim 12 wherein the target epitope is that of an infectious agent.

25. The antibody of claim 12 wherein the target epitope is that of an antibody-producing cell.

```
          10v   20v       30v       40v       50v
022NMVH   QVQLQESGPGLVRPSQTLSLTCTVSGFIFSDNYMYWVRQPPGRGLEWIGTI
          :VQL ESG GLV:P: :L.L:C..SGFIFSDNYMYWVRQ.P.: LEW::TI
022MUVH   EVQLVESGGGLVKPGGSLRLSCVASGFIFSDNYMYWVRQTPEKRLEWVATI
            10^   20^       30^       40^       50^
            60v   70v       80v       90v       100v
022NMVH   SDGGSYTYYPDSVKGRVTMLRDTSKNQFSLRLSSVTAADTAVYYCARGYYR
          SDGGSYTYYPDSVKGR T: RD.:KN:: L::SS:.:.DTA:YYCARGYYR
022MUVH   SDGGSYTYYPDSVKGRFTISRDNAKNNLYLQMSSLKSEDTAIYYCARGYYR
            60^   70^       80^       90^       100^
            110v  120v
022NMVH   YEGAMDYWGQGSLVTVSS
          YEGAMDYWGQG: VTVSS
022MUVH   YEGAMDYWGQGTSVTVSS
            110^  120^
```

FIG. 1A

```
                10v       20v       30v       40v       50v
022KLVH  EVQLVESGGGVVQPGRSLRLSCSSSGFIFSDNYMYWVRQAPGKGLEWVATI
         EVQLVESGGG:V:PG SLRLSC :SGFIFSDNYMYWVRQ:P.K LEWVATI
022MUVH  EVQLVESGGGLVKPGGSLRLSCVASGFIFSDNYMYWVRQTPEKRLEWVATI
                10^       20^       30^       40^       50^
                60v       70v       80v       90v      100v
022KLVH  SDGGSYTYYPDSVKGRFTISRDNSKNTLFLQMDSLRPEDTGVYFCARGYYR
         SDGGSYTYYPDSVKGRFTISRDN:KN.L1.QM.SL11EDT11Y1CARGYYR
022MUVH  SDGGSYTYYPDSVKGRFTISRDNAKNNLYLQMSSLKSEDTAIYYCARGYYR
                60^       70^       80^       90^      100^
               110v   120v
022KLVH  YEGAMDYWGQGTPVTVSS
         YEGAMDYWGQGT:VTVSS
022MUVH  YEGAMDYWGQGTSVTVSS
               110^   120^
```

FIG. 1B

```
                10v        20v        30v        40v        50v
022MUVK  NIVMTQSPSSLAVSAGEKVTMSCKSSQSVLYSSNQKNYLAWYQQKPGQSPK
         :I :TQSPSSL:.S.G::VT::CKSSQSVLYSSNQKNYLAWYQQKPG::PK
022HUVK  DIQLTQSPSSLSASVGDRVTITCKSSQSVLYSSNQKNYLAWYQQKPGKAPK
                10^        20^        30^        40^        50^
                60v        70v        80v        90v       100v
022MUVK  LLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCHQYLSSWT
         LLIYWASTRESGVP.RF:GSGSGTDFTlTISS:Q:ED:A.YYCHQYLSSWT
022HUVK  LLIYWASTRESGVPSRFSGSGSGTDFTFISSLQPEDIATYYCHQYLSSWT
                60^        70^        80^        90^       100^
                110v
022MUVK  FGGGTKLEIK
         FG GTK:EIK
022HUVK  FGQGTKVEIK
                110^
```

FIG. 2

FIG. 3

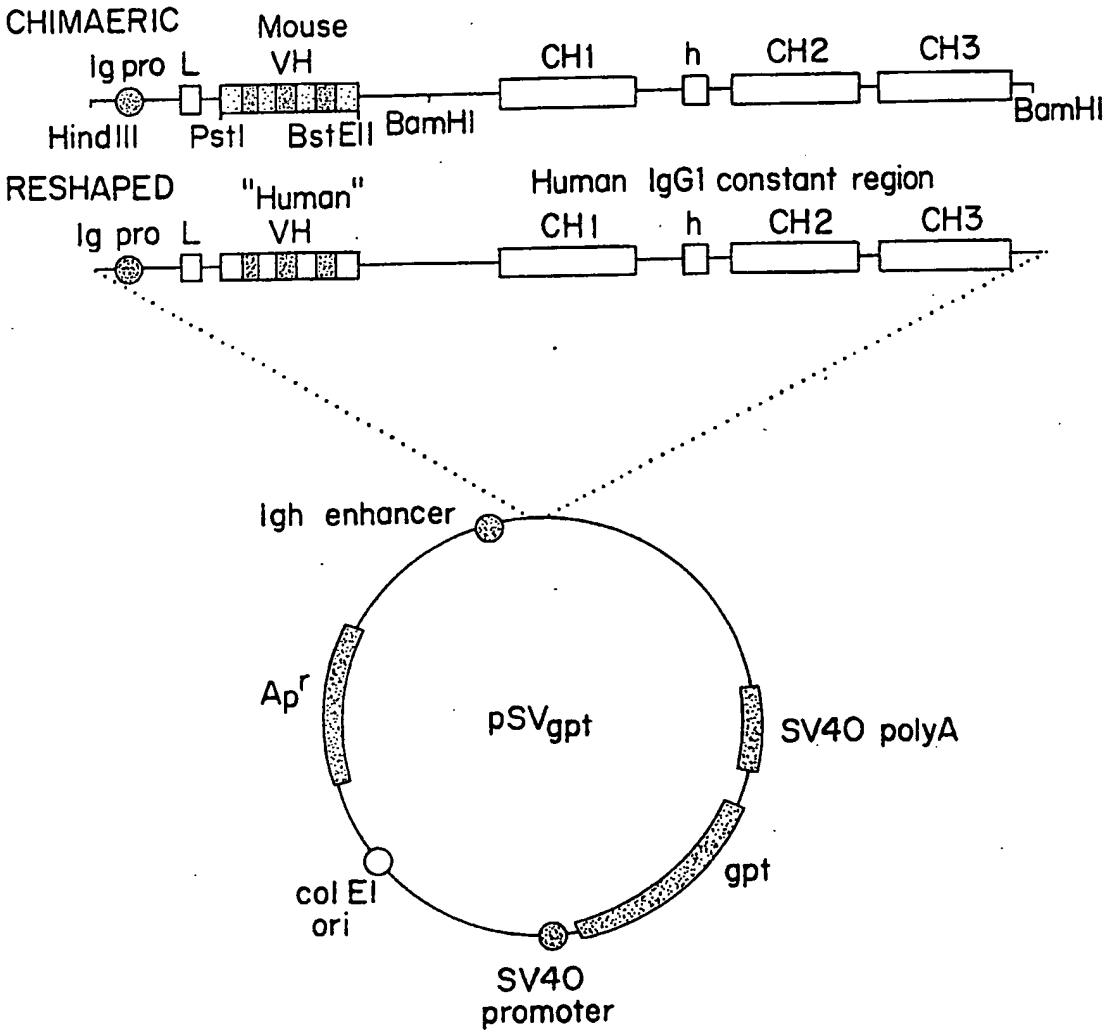

CHIMAERIC
Ig pro L
Mouse VH
CH1
h
CH2
CH3
HindIII
PstI
BstEII
BamHI
BamHI
RESHAPED
"Human" VH
Human IgG1 constant region
Ig pro L
CH1
h
CH2
CH3
Igh enhancer
Ap^r
pSVgpt
SV40 polyA
gpt
col EI ori
SV40 promoter

FIG. 4

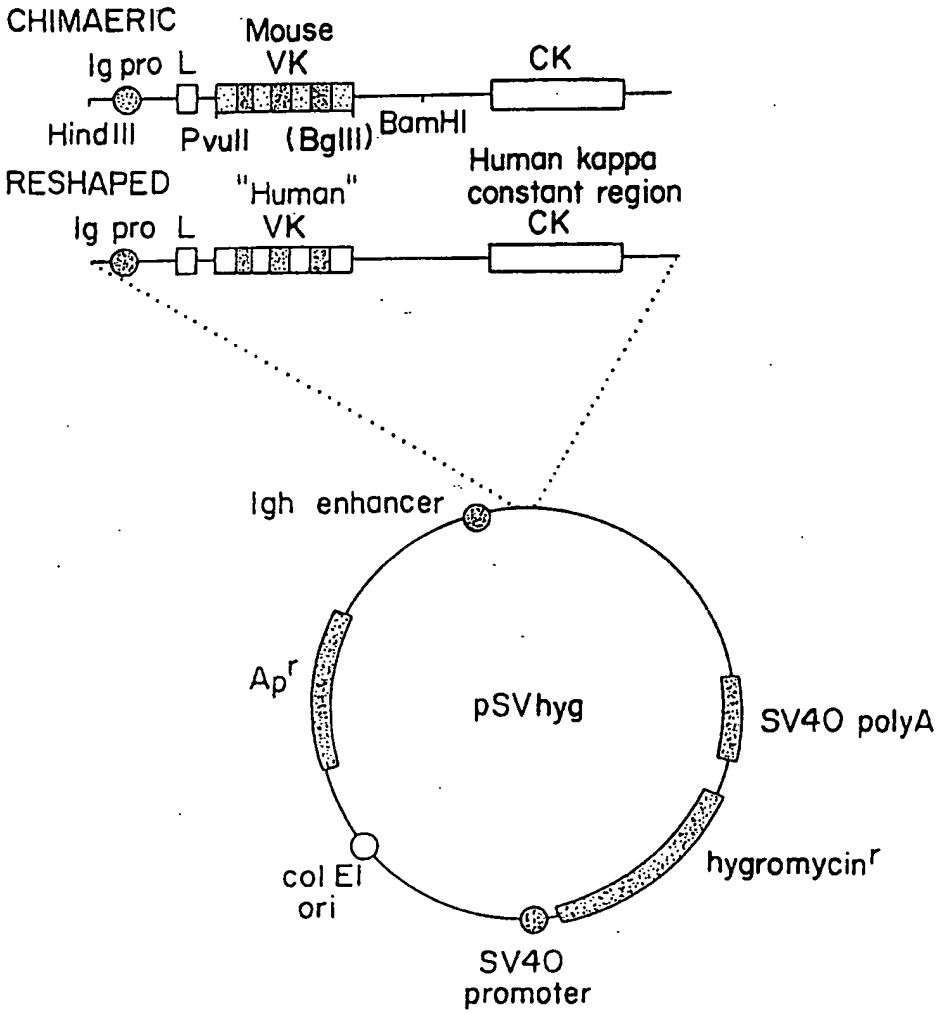
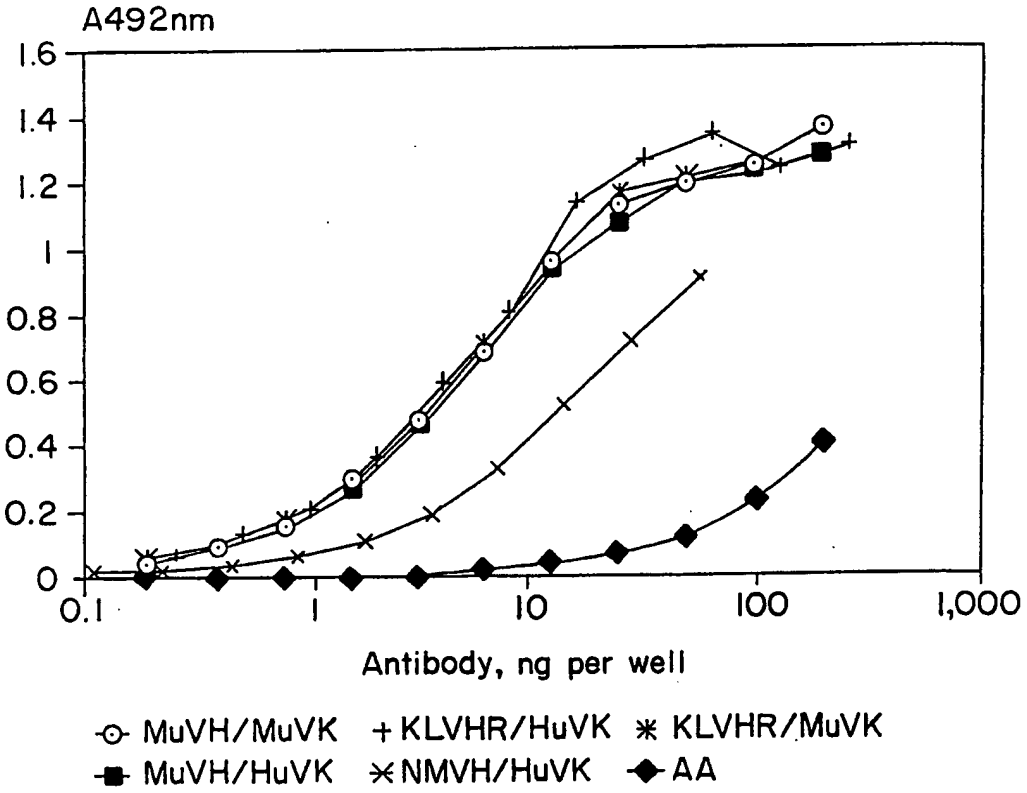

CHIMAERIC
Mouse
VK
Ig pro L
CK
HindIII
PvuII
(BglII)
BamHI
RESHAPED
"Human"
VK
Ig pro L
Human kappa
constant region
CK
Igh enhancer
Ap^r
pSVhyg
SV40 polyA
hygromycin^r
col EI
ori
SV40
promoter

FIG. 5


A492nm
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.1
1
10
100
1,000
Antibody, ng per well
MuVH/MuVK
KLVHR/HuVK
KLVHR/MuVK
MuVH/HuVK
NMVH/HuVK
AA

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/US 93/10384

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12P21/08 C07K15/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12P C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 340 002 (K. TSUJI) 2 November 1989 see claims see example 8 see table 4 ---	1,2
Y	EP,A,0 255 249 (TRUSTEES OF DARTMOUTH COLLEGE) 3 February 1988 cited in the application see the whole document ---	1-25
Y	NATURE vol. 332 , 24 March 1988 , LONDON, GB pages 323 - 327 L. RIECHMANN ET AL. 'Reshaping human antibodies for therapy.' cited in the application see the whole document --- -/--	1-25

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 February 1994	21. 02. 94
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Nooij, F

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GENE vol. 101, no. 2 , 1991 , AMSTERDAM, THE NETHERLANDS pages 297 - 302 A. LEWIS ET AL. 'Immunoglobulin complementarity-determining region grafting by recombinant polymerase chain reaction to generate humanised monoclonal antibodies.' see the whole document	1

1

INTERNATIONAL SEARCH REPORT

information on patent family members

Intern al Application No
PCT/US 93/10384

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0340002	02-11-89	JP-A-	2049597	19-02-90
EP-A-0255249	03-02-88	US-A-	4954617	04-09-90
		AU-B-	605771	24-01-91
		AU-A-	7527187	14-01-88
		CA-A-	1319899	06-07-93
		WO-A-	8800052	14-01-88
		JP-T-	1500195	26-01-89